Exhibit A

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Control of human B-lymphocyte replication

I. CHARACTERIZATION OF NOVEL ACTIVATION STATES THAT PRECEDE THE ENTRY OF G_0 B CELLS INTO CYCLE

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SUMMARY

Tonsillar B lymphocytes of a particularly high buoyant density were prepared essentially free of contaminating monocytes and T cells. When exposed to anti-immunoglobulin, such cells initiated the hydrolysis of inositol phospholipids. This provides a postulated 'dual signal' for growth through the liberation of intracellular calcium stores and the activation of protein kinase C. Nevertheless, neither anti-immunoglobulin nor direct agonists of this bifurcating pathway (respectively, calcium ionophore and the phorbol ester TPA) were capable, when used alone, of driving cells out of G_0 and into RNA synthesis. All three agents did, however, induce two activation antigens at the surface of G_0 B cells, which included CD23, p45 and a lineage-unrestricted antigen identified by the monoclonal antibody BK.19.9. Cells that had been exposed to calcium ionophore, but not those activated with either TPA or anti-immunoglobulin, revealed further change indicated by an increased accessibility of their native DNA for the intercalating dye acridine orange. Cells receiving full mitogenic signals in the form of *Staphylococcus aureus* Cowan Strain I (SAC) or a combination of TPA and calcium ionophore showed the same initial sequelae but continued to enter the cell cycle and progress through to DNA synthesis. The observations identify two phases in the early activation of human B cells, both in terms of various temporal events, and the signals required to promote each activation state. Furthermore, cells receiving complete growth signals were required to transit these activation states before entering the proliferative cycle. Thus, the exit of human B cells from G_0 appears subject to multiple controls that precede those associated with G_1 and later phases of the cell cycle.

INTRODUCTION

The mammalian cell cycle is punctuated by a series of discrete arrests that ensure for normal cells a controlled progression to limited replication and terminal differentiation. For cycling murine B lymphocytes, a number of growth-restriction points have been identified that, in turn, require antigen, monocytes and T cells (or factors derived from them) in order for the cell cycle to be completed (Melchers & Lernhardt, 1985). It is becoming clear, however, again from studies in the murine system, that B cells residing out-of-cycle in G_0 can achieve a level of activation that is transitional to entry into the growth cycle proper (Cambier *et al.*, 1985; Klaus *et al.*, 1984). One feature of such cells is an accelerated DNA synthesis in response to appropriate secondary signals.

An important mechanism of signal transduction in lymphocytes is the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2), which gives rise to diacylglycerol and inositol trisphosphate. These metabolic intermediates are thought to provide a so-called 'dual pathway' for growth by acting as second messengers to activate protein kinase C and to liberate intracellular calcium stores, respectively (Berridge, 1984). Direct evidence for the involvement of this bifurcating pathway in lymphocyte activation has been obtained for cells of both T and B lineages. Thus, ligation of surface antigen receptors has been shown to initiate inositol phospholipid metabolism, while combinations of the phorbol ester TPA and calcium ionophore (direct agonists of the separate arms of the pathway) are mitogenic for resting lymphocytes (Bijsterbosch *et al.*, 1985; Imboden & Stobo, 1985; Truneh *et al.*, 1985; Guy *et al.*, 1985a, b).

In the study reported here, we have approached the possibility that, as with murine B cells, distinct levels of activation may be available to human B lymphocytes before they enter the cell cycle. In order to investigate this, we have used highly purified resting B cells and activators that either initiate hydrolysis of the inositol phospholipids or mimic the secondary

Abbreviations: CaI, calcium ionophore; IP_3, inositol trisphosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; SAC, *Staphylococcus aureus* Cowan Strain I; TPA, 12-0-tetradecanoyl-phorbol-13-acetate.

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messengers subsequently generated. By using a multi-parameter analysis of activation, we have been able to identify three subcompartments within G_0, each associated with a distinct phenotype and potential growth arrest. Thus, the controls, on entry of the resting cell into an active growth cycle, appear much greater than had previously been considered for either murine or human B lymphocytes.

MATERIALS AND METHODS

Activators used

Staphylococcus aureus Cowan Strain I was obtained from Calbiochem-Behring (Cambridge), ionomycin was from Calbiochem (La Jolla, CA) and TPA was from Sigma (Poole, Dorset). Anti-immunoglobulin was a $F(ab')_2$ IgG preparation from goat antiserum to human μ chains (Cappel, Cochranville, PA). Agents were used at concentrations indicated in the text.

Preparation of resting B cells

The isolation of B cells from tonsils obtained at routine tonsillectomy by negative selections of cells binding modified sheep erythrocytes has been described in detail previously (Gordon, Guy & Walker, 1985). Cells banding below a 62·5% Percoll (Pharmacia, Uppsala, Sweden) gradient constituted the ultra-high buoyant density population used in this study.

Detection of surface antigens

The presence of surface antigens was measured by a sensitive indirect rosetting assay using sheep erythrocytes coated with sheep antibodies to mouse immunoglobulins as the indicator cells and the following mouse monoclonal antibodies as the initial layer: BK19.9 recognizing a ubiquitous proliferation antigen structurally similar to, but serologically distinct from, the transferrin receptor (Gatter *et al.*, 1983); MHM6 identifying the CD23, p45 B-cell restricted activation antigen (Rowe *et al.*, 1982); 11EF7, a novel antibody raised in the Department and recognizing a late B-cell activation antigen; A2, which recognizes the transferrin receptor (a gift of Dr A. Bernard, Institut Gustav-Roussy, Villejuif). Positive cells were defined as those forming rosettes with more than five attached erythrocytes.

Hydrolysis of inositol phospholipids

The hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) was measured by the specific accumulation of [^{3}H]inositol into inositol trisphosphate at the indicated times. B cells were labelled for 4 hr in Hanks' HEPES medium containing 5 μCi [^{3}H]inositol per 10^6 cells, washed twice then reseeded in Costar plate wells (0·6 ml) at $3–4 \times 10^6$ cells per well. Treatments were added in 30 μl of medium, and reactions were terminated and the products analysed on Dowex resin columns (formate type) by the method of Berridge, Downes & Hanley (1982).

DNA and RNA synthesis

B cells, 5×10^5, were cultured at 37° in 200 μl of RPMI-1640 supplemented with 10% fetal calf serum (screened non-mitogenic batch), 2 mM L-glutamine and 5×10^{-5} M 2-mercaptoethanol. At the times indicated in the text, 50 μl of either [^{3}H]uridine or [^{3}H]thymidine, both at 0·01 mCi/ml, were added to the cultures, which were pulsed for the periods shown. Plates were frozen prior to harvesting and the amounts of incorporated radioactivity subsequently determined.

Flow cytometric analysis

The RNA and DNA content of individual cells were assessed according to the method of Darzynkiewicz *et al.* (1980). Briefly, cells were taken from culture, washed in serum-free physiologically buffered saline, permeablized by the addition of Triton X-100, and stained with acridine orange (Polysciences Inc., Warrington, PA) at a final dye concentration of 13 μM. Cells were kept on melting ice and assessed within 30 min for green (515–575 nm) and red (600–650 nm) fluorescence emission on a FACS IV flow cytometer (Becton-Dickinson, Mountain View, CA) fitted with appropriate filters. Intercalation of the dye with DNA yields maximum emission at 530 nm, while interaction with RNA emits maximally at 640 nm. Values given are based on data collected from 50,000 cells. The levels of forward (narrow angle) and 90° scattered light were collected simultaneously for each run. In addition, unfixed viable cells were assessed in parallel for the degree of forward light scattered.

RESULTS

Heterogeneity of 'resting' tonsillar B cells

In an earlier study, one of us (JG) noted that tonsillar B cells of high buoyant density were heterogeneous with regard to certain activation-related antigens (Aman *et al.*, 1985). Thus, around 20% of 'resting' B cells were positive for the BB-1 activation antigen, while up to 35% were BB-2-positive by indirect immunofluorescence. Further heterogeneity in these populations was indicated by a variable proportion of cells staining positively with B2, HB-2, the 'Burkitt lymphoma antigen' identified by 38.13 and the number of cells positive for IgM and IgD (Aman *et al.*, 1985). Using the sensitive indirect rosetting technique, we have now found that as many as 80% of the cells collected below a 57·5% Percoll gradient can be positive for the BB-1 antigen, although the precise number varied among different tonsil preparations.

In the present study, we have investigated B-cell activation status using the following monoclonal antibodies: MHM6, which recognizes the CD23, p45 antigen [also referred to as BLAST-2 or EBVCS (Thorley-Lawson *et al.*, 1985)]; BK19.9 describing a lineage-unrestricted 'proliferation' antigen, which is structurally similar to transferrin receptor but is serologically distinct (Gatter *et al.*, 1983); A2, which recognizes the transferrin receptor; 11EF7, a novel monoclonal antibody identifying a B-cell restricted antigen appearing relatively late in activation. B-cell populations collected below 57·5% Percoll invariably contained less than 2% of cells expressing either the transferrin receptor or the 11EF7-defined antigen. By contrast, the number of cells positive for either BK19.9 or MHM6 varied between 2% and 47%, and 4% and 56%, respectively. Further fractionation of cells revealed that populations banding below 62·5% Percoll were always less than 5%, and usually below 2%, positive for these two markers of activation.

Analysis of cell cycle status showed that >95% of cells collected below 57·5% Percoll posessed a G_0 content of RNA. However, as illustrated in Fig. 1a, it was apparent that the level of fluorescence emission from acridine orange bound to DNA was heterogeneous. Furthermore, among individual tonsils, the relative proportion of cells displaying an apparently high DNA content to those giving a low level emission varied considerably. It was found that by collecting the cells that banded below a

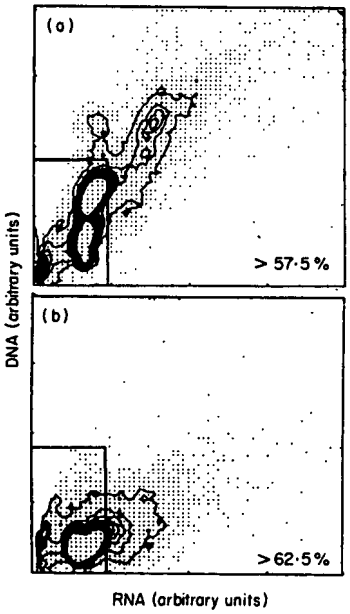

Figure 1. Cell cycle profile of 'resting' tonsillar B cells. B cells freshly isolated from tonsils and subjected to density sedimentation on Percoll gradients (percentages indicated) were analysed for their DNA versus RNA content by flow cytometric analysis of acridine orange stained cells. The results of 50,000 cells analysed are represented as a two-dimensional contour plot of DNA (green) *vs* RNA (red) fluorescence. The G_0 compartment, determined by blocking G_1 entry of activated cells in the presence of actinomycin D, is indicated by the boxed area in the bottom left-hand corner.

62·5% Percoll gradient, a more homogeneous DNA profile was obtained, with a majority of cells displaying a low level fluorescence emission from acridine orange bound to DNA (Fig. 1b). These 'ultra-high density' tonsillar B cells, essentially devoid of any detectable marker of activation, were used as the resting populations in this study and represented 10–30% of total B cells. Such preparations were found to contain less than 1:500 cells positive for non-specific esterase or forming rosettes with modified sheep erythrocytes. Examination with a series of pan-monocyte and pan-T monoclonal antibodies confirmed this essential absence of contaminating cells.

Hydrolysis of inositol phospholipids, cell cycle entry and mitogenesis

We have recently shown that killed particles of SAC, which act as a complete mitogen, trigger the hydrolysis of inositol phospholipids in resting tonsillar B cells (Guy *et al.*, 1985b). In this study, we compared soluble anti-immunoglobulin with SAC in its ability to generate inositol trisphosphate (IP_3), a major product of PIP_2 hydrolysis. We found that a concentration of anti-immunoglobulin that primes resting B cells optimally for DNA synthesis in response to T-cell supernatants (Gordon, Guy & Walker, 1986) was as effective as SAC in initiating the hydrolysis of PIP_2 as measured by the accumulation of IP_3 during the first 2 min (Table 1). However, whereas SAC maintained its influence, the hydrolysis of PIP_2 by anti-immunoglobulin was more transitory, beginning to wane by 5 min and essentially ceasing after 10 min as suggested by the plateau in the accumulated levels of IP_3 (Table 1). Increasing the

Table 1. Inositol trisphosphate accumulation in B cells triggered through their antigen receptors

	[^{3}H]IP_3 accumulation (d.p.m. per 10^7 cells)				
	No. minutes post-activation*				
	1	2	5	10	30
Control	21	25	35	48	55
Anti-Ig†	92	150	175	205	235
SAC	105	185	305	454	1008

* Results given as mean of duplicate determinations, which never varied by more than 20% of each other. The experiment was performed on three separate occasions yielding essentially identical results each time.

† Anti-Ig = anti-immunoglobulin [$(F(ab')_2$ preparation of IgG fraction]. Reagents used at the concentrations indicated in text.

concentration of antibody failed to either increase or prolong its effect (data not shown). These findings suggest that it is necessary to present a highly cross-linked persistent signal to B-cell antigen receptors in order for the hydrolysis of inositol phospholipid to be sustained. Agonists of the pathways generated through PIP_2 hydrolysis, at concentrations where together

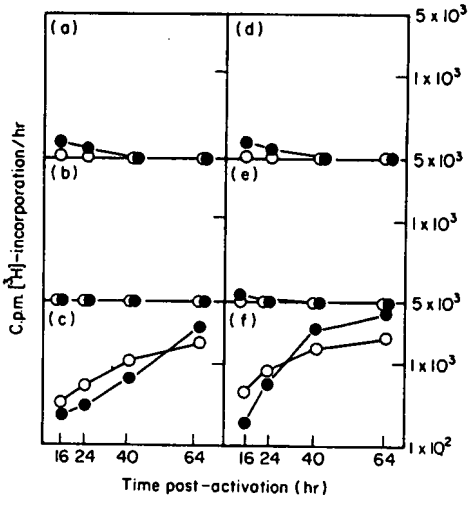

Figure 2. RNA and DNA synthesis following B-cell activation. B cells collected below 62·5% Percoll were exposed to the following agents: (a) control; (b) soluble $F(ab')_2$ anti-immunoglobulin (15 μg/ml); (c) SAC (1:20,000); (d) TPA (0·1 ng/ml); (e) ionomycin (0·8 μg/ml); and (f) a combination of (d) and (e). Cultures were pulsed with 0·5 μCi of [^{3}H]uridine (○) or [^{3}H]thymidine (●) over the time-intervals indicated. The results are expressed as the hourly rate of incorporated radioactivity given as the mean of triplicate determinations, which did not vary by more than 5%.

they provide a full growth signal for resting B cells (Guy *et al.*, 1985a), were found not to influence its breakdown as judged by the accumulation of IP_3 (data not shown).

Neither anti-immunoglobulin (15 μg/ml), the calcium ionophore ionomycin (0·8 μg/ml) nor the phorbol ester TPA (0·1 ng/ml) was able to drive the highly purified resting B cells into cycle, as shown by their failure to increase RNA synthesis at any time over 64 hr (Fig. 2). By contrast, not only SAC, but also a combination of the phorbol ester and calcium ionophore, were highly efficient at inducing both RNA and, later, DNA synthesis. This suggests that cell cycle entry and mitogenesis are dependent on a chronic stimulation of both pathways generated through the hydrolysis of PIP_2. We should stress that the inability of the minimal activators to promote even RNA synthesis was strictly dependent on using both highly purified populations and cells of ultra-high density.

Induction of activation antigens on resting B cells

Exposure of the resting B cells to any one or a combination of the activators studied led to the appearance of the antigens defined by MHM6 and BK19.9. Their induction was extremely rapid, being first detected between 3 hr and 4 hr and present on a large number of cells by 6 hr (Table 2). Thus, the appearance of these antigens was independent of cells entering the cell cycle. In contrast, only those activators that increased RNA synthesis, and thus prompted cell cycle entry, were capable of inducing the appearance of the transferrin receptor and the B-cell restricted antigen defined by 11EF7 (Table 2). Furthermore, these antigens appeared later than those defined by MMH6 and BK19.9 at a time coinciding with the initiation of high level DNA synthesis.

Flow cytometric analysis of B-cell activation

Flow cytometric analysis of acridine orange stained cells allowed a simultaneous four-parameter read-out of RNA content, DNA content, 90° scatter and forward (narrow angle) scatter. No change in any of these parameters was observed at 6 hr, irrespective of the signal the cells received, and even though the expression of BK19.9 and MHM6 antigens was nearly complete. The lack of change in forward scatter when viable cells were analysed indicates that the appearance of these antigens preceded any increase in cell size. However, by 12 hr, and notably between 18 hr and 24 hr, appreciable changes in both 90° scatter and fluorescence emission from acridine orange bound to DNA were observed for B cells activated with ionomycin, either alone or in combination with TPA, and with SAC (Fig. 3). When B cells were activated with SAC or ionomycin together with TPA, these changes preceded subsequent increases in RNA content and DNA synthesis. When ionomycin was used alone, increased DNA staining and 90° scatter occurred without any accompanying change in RNA content over the whole period of observation. These early changes in DNA staining and 90° scatter were thus independent of cell cycle entry and of actual DNA synthesis. Furthermore, the level of DNA staining achieved by 24 hr did not reach S-phase DNA content (determined by blocking proliferating cells at the G_1/S transition with hydroxyurea). B cells exposed to TPA alone, at the dose that synergized optimally with ionomycin to promote full cell cycle progression, showed no increase in any of the parameters revealed by flow cytometry over the whole of the study. Exposure of the cells to soluble anti-immunoglobulin, which like TPA above induced BK19.9 and MHM6 expression, also resulted in no overall increase in DNA emission and 90° scatter (Fig. 3).

Inspection of contour plots of RNA versus DNA emissions at 44 hr revealed that cells that had received a complete mitogenic signal increased their level of DNA staining to a level comensurate with that of cycling cells before they entered G_1 (Fig. 4). Similarly, it can be seen that activators that failed to take resting cells to their highest level of DNA staining within G_0 were incapable of promoting entry into G_1.

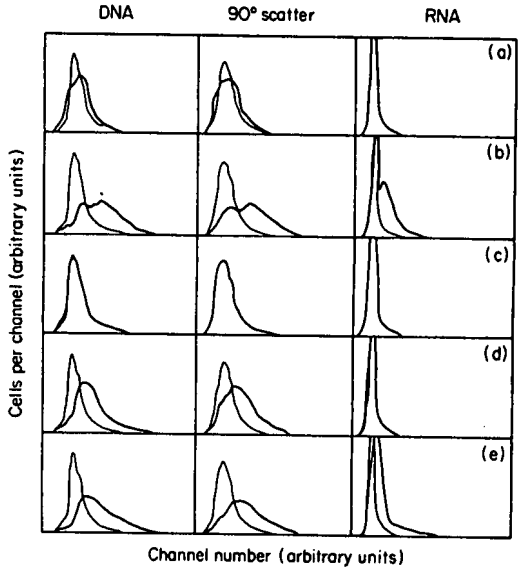

Figure 3. Early changes in flow cytometry parameters. All data are represented as histogram plots taken from 50,000 cells analysed 24 hr post-activation: (a) anti-immunoglobulin; (b) SAC; (c) TPA; (d) ionomycin; and (e) a combination of (c) and (d). Heavy lines represent test samples, while faint lines indicate control emissions. Channel number is represented on a linear scale.

Table 2. Induction of activation antigens on B cells

	% cells rosette positive:							
	BK19.9		MHM6		A2		11EF7	
	6 hr	24 hr	6 hr	24 hr	16 hr	40 hr	16 hr	40 hr
Control	4	1	2	2	1	3	0	0
Anti-Ig*	33	58	40	60	4	4	0	2
SAC	42	63	46	57	7	45	4	38
TPA	30	49	42	56	4	2	1	0
CaI†	40	37	42	40	3	5	2	1
TPA+CaI	71	79	66	65	11	59	0	46

Reagents used at concentrations indicated in text.
* Anti-Ig = anti-immunoglobulin.
† CaI = calcium ionophore.

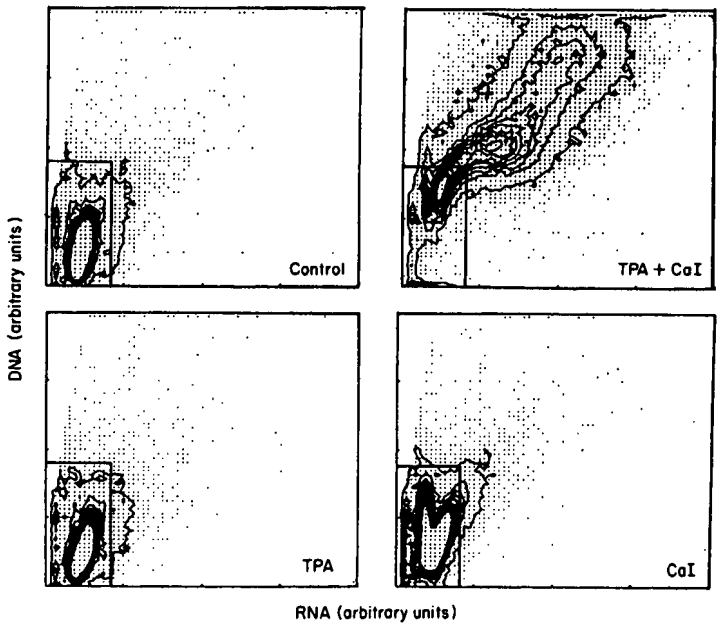

Figure 4. Cell cycle progression of activated B cells. As in Fig. 1, but this time cells were analysed 44 hr post-activation with the agents indicated. The G_0 compartment is again boxed. Note the large number of cells now in S-phase (indicated by proportional increases in both DNA and RNA) when TPA and ionomycin are used in combination, by contrast with cells exposed to either activator alone remaining in G_0.

DISCUSSION

These studies have identified two stages in the early activation of human B cells that precede their entry into the cell cycle. The first phase is characterized by the appearance of two surface antigens that are absent on the surface of truly resting cells. Both antigens have been linked to activation. CD23, p45 has been claimed to be the first B-lineage restricted antigen to appear on B cells following their infection with Epstein–Barr virus (Thorley-Lawson & Mann, 1985). Our findings are compatible with this notion, and extend it by demonstrating that any minimal activator of B cells will induce the expression of this antigen. Furthermore, we have shown that the induction of CD23, p45 is extremely rapid, being found on a large number of cells by 6 hr following activation. Coincident with the appearance of CD23, p45 was the induction of the lineage-unrestricted antigen defined by BK19.9. Although initially suspected to share identity with the transferrin receptor, it is now clear that the antigen recognized is quite distinct (Gatter *et al.*, 1983, and our unpublished observations). Its function remains unknown, but its early appearance on activation, its wide tissue distribution and its high expression in malignancy suggest that this antigen may have an essential role in cell growth and survival.

The second phase of activation was identified by an increased ability of native DNA to bind intercalating acridine orange. This was again independent of cell cycle entry, preceding the exit of cells from G_0. The precise nature of this early change is unclear, but in the absence of *de novo* DNA synthesis, increased emission from acridine orange bound to DNA presumably reflects easier access of DNA intercalating sites for the dye. Precedents exist in other systems to suggest that this, in turn, reflects a decondensing of chromatin structure prior to new gene transcription (Baserga & Nicolini, 1976). Such a change in chromatin structure might also account for the accompanying increase in light deflected at 90°. While similar changes in DNA stainability have been observed for density-arrested fibroblasts (Nicolini *et al.*, 1977), they have not been seen in lymphocytes unless the DNA was first denatured (Darzynkiewicz *et al.*, 1977). This apparent discrepancy between our studies and previous studies of peripheral blood T lymphocytes can be explained by our policy of isolating the minority of B cells that can be considered as truly quiescent. Indeed, we noted that, rather than displaying hyper-staining of DNA during early activation, cells moved from a low level of staining to a level comensurate with cells actively participating in the growth cycle.

Of the minimal activators (i.e. those that were not of themselves mitogenic), only the calcium ionophore promoted the second phase of activation. Furthermore, this was reached without cells continuing to leave G_0 and enter the cycle. The observation that some activators prompted cells into the first or second levels of activation but no further suggests that these states represent control points in the growth of B lymphocytes that precede those identified for G_1 and later phases of the cycle (Melchers & Lernhardt, 1985). Furthermore, cells receiving complete mitogenic signals were required to proceed sequentially through the activation levels in G_0 before participating in the growth cycle proper. A transitional activation state between G_0 and G_1 has been proposed for murine B cells, and has been termed by some 'G_0^*', and by others 'G_{1T}' (Cambier *et al.*, 1985; Klaus, Bijsterbosch & Holman, 1985). For murine B cells this state is associated with membrane depolarization, increased expression of I-A and an accelerated entry of cells into S-phase when exposed to appropriate second signals. Activators of murine B cells capable of achieving a transitional activation state include calcium ionophores, phorbol ester, concanavalin

A, whole antibody to surface immunoglobulin, and B-cell stimulatory factor 1 (Hawrylowicz, Keeler & Klaus, 1984; Klaus *et al.*, 1984; Hawrylowicz & Klaus, 1984; Klaus *et al.*, 1985; Rabin, O'Hara & Paul, 1985; Cambier *et al.*, 1985). Our studies show that more than one activation state is available to human G_0 B cells, and that each level of activation is associated with a distinct phenotype.

The present study confirms a role for the receptor-mediated hydrolysis of inositol phospholipids in B-cell activation. Much evidence has emerged to implicate this pathway in the triggering of murine B lymphocytes (Grupp & Harmony, 1985; Bijsterbosch *et al.*, 1985), and we have recently shown that human B cells respond to SAC via PIP_2 hydrolysis (Guy *et al.*, 1985b). Furthermore, we and others have demonstrated that agonists of the bifurcating pathway generated through PIP_2 hydrolysis, when applied in combination, drive human B cells through the cell cycle to DNA synthesis and replication (Guy *et al.*, 1985a; Clevers *et al.*, 1985). From the present study, it is clear that the ability of deeply quiescent B cells to achieve one activation state and progress to the next is dependent more on the magnitude and duration of the initiating signal than its quality. Thus, while both SAC and soluble anti-immunoglobulin were capable of initiating PIP_2 hydrolysis, only SAC maintained its influence. With anti-immunoglobulin, the effect was transitory and resulted in cells reaching the first activation level only. Similarly, while it is known that anti-immunoglobulin signals a rise in intracellular free Ca^{2+} (Pozzan *et al.*, 1982), calcium ionophore, which presumably supplies a more sustained Ca^{2+} elevation, was able to take cells further, through to the second level of activation within G_0. Recent studies in the murine system, showing that the initiation of inositol phospholipid metabolism is not, of itself, sufficient for mitogenesis, are consistent with our observations on human B lymphocytes (Bijsterbosch & Klaus, 1985). Finally, in unpublished experiments, we have found that by increasing the concentration of phorbol ester, B cells can be taken to increasingly later stages of activation in both G_0 and G_1. Indeed, very high concentrations of TPA are capable of inducing at least a proportion of resting B cells to enter S-phase (Guy *et al.*, 1985a). It is of interest that some activators (e.g. SAC and TPA alone) triggered only a proportion of cells, whereas a combination of TPA and calcium ionophore stimulated essentially all the cells. The possibility that our quiescent B cells remain heterogeneous with regards to lineage/differentiation markers is currently under investigation.

By analogy with the nomenclature adopted for the G_1 phase of the cycle (Darzynkiewicz *et al.*, 1980), we would like to suggest that the newly identified subcompartments of human B-cell G_0 are termed 'G_{0Q}', 'G_{0A}' and 'G_{0B}'. G_{0Q} represents B cells in a true, deep quiescence, totally devoid of activation antigens and displaying highly condensed nuclear chromatin. G_{0A} and G_{0B} constitute the first and second levels of activation identified, respectively, by the appearance of new surface antigens and an increased DNA stainability. The fact that the phenotypic attributes of all three subcompartments could be found among freshly isolated tonsillar cells indicates that these activation states are not simple artefacts of the *in vitro* system. The concepts that have emerged from this study are embodied in the model presented in Fig. 5. An appreciation of the model provides a firm basis on which to explore the action of cell contacts, soluble factors and the receptors that influence human B cells at different stages of the cell cycle. In this regard, it is interesting to speculate on the relationship between CD23, p45 in man and the Lyb2 antigen in mouse, which is also a 45,000 molecular weight protein and which transmits growth signals to 'resting' B cells (Subbarao & Mosier, 1984).

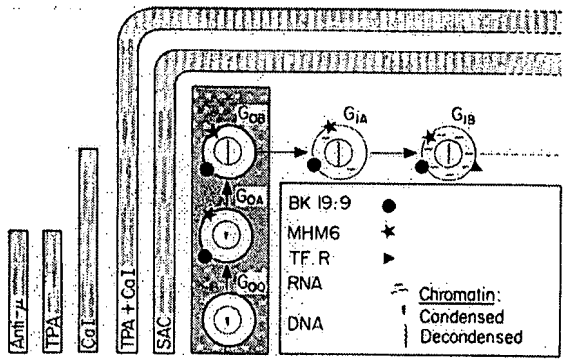

Figure 5. A model for G_0 transition by activated B cells. The model highlights the phenotypic characteristics of the new G_0 subcompartments identified for human B cells and their transit by the activators studied (see Discussion). CaI = calcium ionophore; anti-μ = anti-immunoglobulin. The B-cell restricted antigen identified by 11EF7 is not included in the scheme, but the similarity of its kinetics with the transferrin receptor (TF.R) suggests that it appears late in G_1. The changes observed in DNA stainability and 90° scatter are taken to represent alterations in chromatin structure. The shaded bars represent the limit of action of the stimulus shown.

ACKNOWLEDGMENT

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